EXAMPLE 152: Proliferation of Rat Utricular Supporting Cells (Assay 54)

This assay shows that certain polypeptides of the invention act as potent mitogens for inner ear supporting cells which are auditory hair cell progenitors and, therefore, are useful for inducing the regeneration of auditory hair cells and treating hearing loss in mammals. The assay is performed as follows. Rat UEC-4 utricular epithelial cells are aliquoted into 96 well plates with a density of 3000 cells/well in 200 μl of serum-containing medium at 33°C. The cells are cultured overnight and are then switched to serum-free medium at 37°C. Various dilutions of PRO polypeptides (or nothing for a control) are then added to the cultures and the cells are incubated for 24 hours. After the 24 hour incubation, ^{3}H-thymidine (1 μCi/well) is added and the cells are then cultured for an additional 24 hours. The cultures are then washed to remove unincorporated radiolabel, the cells harvested and Cpm per well determined. Cpm of at least 30% or greater in the PRO polypeptide treated cultures as compared to the control cultures is considered a positive in the assay.

The following polypeptides tested positive in this assay: PRO1340.

EXAMPLE 153: Chondrocyte Proliferation Assay (Assay 111)

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to induce the proliferation and/or redifferentiation of chondrocytes in culture. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of various bone and/or cartilage disorders such as, for example, sports injuries and arthritis.

Porcine chondrocytes are isolated by overnight collagenase digestion of articular cartilage of the metacarpophalangeal joint of 4-6 month old female pigs. The isolated cells are then seeded at 25,000 cells/cm^2 in Ham F-12 containing 10% FBS and 4 μg/ml gentamycin. The culture media is changed every third day and the cells are reseeded to 25,000 cells/cm^2 every five days. On day 12, the cells are seeded in 96 well plates at 5,000 cells/well in 100μl of the same media without serum and 100 μl of either serum-free medium (negative control), staurosporin (final concentration of 5 nM; positive control) or the test PRO polypeptide are added to give a final volume of 200 μl/well. After 5 days at 37°C, 20 μl of Alamar blue is added to each well and the plates are incubated for an additional 3 hours at 37°C. The fluorescence is then measured in each well (Ex:530 nm; Em: 590 nm). The fluorescence of a plate containing 200 μl of the serum-free medium is measured to obtain the background. A positive result in the assay is obtained when the fluorescence of the PRO polypeptide treated sample is more like that of the positive control than the negative control.

The following PRO polypeptides tested positive in this assay: PRO1265, PRO1412, PRO1347, PRO1279, PRO1410 and PRO1474.

EXAMPLE 154: Inhibition of Heart Neonatal Hypertrophy Induced by LIF+ET-1 (Assay 74)

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to inhibit neonatal heart hypertrophy induced by LIF and endothelin-1 (ET-1). A test compound that provides a positive response in the present assay would be useful for the therapeutic treatment of cardiac insufficiency diseases or disorders characterized or associated with an undesired hypertrophy of the cardiac muscle.

Cardiac myocytes from 1-day old Harlan Sprague Dawley rats (180 μl at 7.5 x 10^4/ml, serum <0.1, freshly isolated) are introduced on day 1 to 96-well plates previously coated with DMEM/F12 + 4%FCS. Test